The universal response to stress is mediated by the expression of heat shock proteins (hsp), which are involved in repair and recovery from the insult. In addition, their presence provides protection from subsequent insults, which has been coined stress tolerance. The cytoprotective role of hsp has been associated with their chaperone function within the cytosol. However, hsp were recently found outside cells where they acted as signaling molecules directed at activating the immune system to avoid the propagation of the insult. Hsp70, the major inducible form of the hsp family, does not contain any consensus secretory signal that predicts its secretion via the ER-Golgi pathway. Therefore, it is likely that the export of Hsp70 to the extracellular environment is mediated by the propose that Hsp70 is exported by a novel mechanism initiated by the translocation of the protein into the plasma membrane and is released associated with vesicles called export or extracellular vesicles (ECV). To test this hypothesis, we developed a liposome insertion assay using pure recombinant Hsp70. We found that Hsp70 insertion into lipid membranes was spontaneous and specific for negatively charged lipids, such as phosphatidylserine and phosphatidylglycerol. In contrast, positive or neutral lipids did not support membrane insertion. We also found that less fluid lipid environments highly favored membrane insertion, which resembles in vivo observations indicating the presence of Hsp70 in lipid rafts of cells. In summary, our observations support the hypothesis that membrane insertion is the first stage of secretion of Hsp70 into the extracellular environment in the form of vesicles. We speculate that hsp70-ECV may result in a robust activation of the immune system that is part of the systemic response to stress.
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2757-Plat
Probing the Structural Origins of Unusually Strong Target Membrane Affinity of Synaptotagmin 7 C2A and C2Ab Domains
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Synaptotagmin (Syt) proteins serve as Ca^{2+} sensitive triggers in many exocytotic pathways, with the seventeen different human isoforms active in various cell types. Syt proteins contain two C2 domains, C2A and C2B, which bind membranes in response to Ca^{2+} to drive vesicle fusion. Much is known about the biophysical mechanism of function for Syt1, which triggers fusion for rapid neurotransmitter secretion, but less mechanistic information is available for other isoforms. Syt7 typically operates in slower pathways requiring smaller peak Ca^{2+} concentrations, and its C2 domains are known to bind membranes with a much higher Ca^{2+} sensitivity compared to Syt1. using kinetic and equilibrium fluorescence measurements of C2A domain docking to synthetic liposomes approximating the lipid composition of physiological membranes, we report that the differences between the two isoforms include kinetic and solute effects consistent with much greater hydrophobic membrane contact for Syt7 C2A. A strong hydrophobic contribution to the membrane docking mechanism of Syt7 C2A stands in contrast to the known electrostatic membrane interaction of Syt1 C2A, and is somewhat surprising given the 90% conserved amino acid polarity between the two domains. In order to test our proposed hydrophobic docking model for Syt7 C2A and probe its structural origins, we use a combination of site-directed mutagenesis, equilibrium and kinetic protein-membrane docking assays, and electron paramagnetic resonance-based depth measurements. In addition, single-molecule measurement of protein lateral diffusion on supported lipid bilayers is used to report on contributions of intra- and intermolecular protein-protein contact to the membrane-docked states of individual C2 domains and C2AB tandems. The results are interpreted to provide information on the structural origins of differences in function between these two isoforms.

Platform: Membrane Receptors & Signal Transduction II

2758-Plat
Enzymatic Activity of GPCRs: Reductase Activity of β2 Adrenergic and Histamine Receptors
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The GPCRs β2 Adrenergic Receptors and Histamine Receptors have been shown to have extracellular enhancement of their receptor activity by ascorbate. Ascorbate binds on the 2nd extracellular loop and shifts the agonist dose-response curve approximately 0.5 log units to the left. When present with ascorbate, receptor-membrane preparations significantly reduced the rate of ascorbate oxidation. As the molar concentration of ascorbate greatly exceeded that of the receptors, one-to-one binding is not possible, and the presences of receptor reductase activity was suspected. In the studies below, ascorbate but not its oxidation product DHA is shown to absorb at 265 nm using both UV spectroscopy (UVS) and capillary electrophoresis (CE). Ascorbate oxidation is shown by the disappearance of ascorbate absorbance. DHA reduction is shown by the appearance of ascorbate. The equilibrium constant for DHA/Asc was found to be 49: 10 mM DHA produced 20 μM Asc at equilibrium. using UVS (AR) and CE (HR), both β2AR and HR increase the DHA reduction rate by 40-50% at 20 mM phosphate. The rate of DHA reduction was found to be dependent on both the phosphate concentration and specifically on the presence of HPO_4^{2-}. The net reduction of DHA is DHA + 2 HPO_4^{2-} ↔ Asc + 2 PO_4^{3-} with the reaction enzymatically enhanced by β2AR and HR. At physiological phosphate concentrations of 1 mM, the fractional increase in DHA reduction due to GPCRs may be even greater than that measured at 20 mM phosphate, as the reduction rate for 1 mM phosphate alone is very low. These observations may be an important addition to GPCR function and to evolutionary theories of reduction in the pre-biotic world.

2759-Plat
Conformational Entropic Maps of Functional Coupling Domains in GPCR Activation: A Case Study of β2 Adrenergic Receptor
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Enthalpic and entropic changes during GPCR activation are poorly understood. Based on the recent solved structures, researchers in the GPCR structural biology field have proposed several 'local activating switches' that consisted of a few number of conserved residues, but have long ignored the collective dynamical effect (conformational entropy) of a domain composed of an ensemble of residues. A new paradigm has been proposed recently that a GPCR can be viewed as a composition of several functional coupling domains, each of which undergoes order-to-disorder or disorder-to-order transitions upon activation. Here we identified and studied these functional coupling domains by comparing the local entropy changes of each residue between the inactive and active states of the β2 adrenergic receptor from molecular dynamics simulation. We found that agonist and G-protein binding increases the heterogeneity of the entropy distribution in the receptor. This new activation paradigm in terms of receptor entropic maps provides a novel way to design functionally biased mutants and may also assist in the identification of allosteric sites in GPCRs.

2760-Plat
Molecular Dynamics Simulations of the Activated Cannabinoid Receptor Subtype 2/Gi Protein Complex
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The cannabinoid receptor subtype 2 (CB2) is a class A (rhodopsin-like) G-protein-coupled receptor (GPCR) that is highly expressed in the immune system (Galiegue et al., 1995) and signals via Gi proteins (Glass and Northup, 1999). The GPCR signaling cascade begins with agonist binding to an inactive receptor, causing conformational changes that activate the receptor. In previous work, we used molecular dynamics simulations to study the activation of the CB2 receptor, by the endogenous ligand, 2-arachidonoylglycerol (2-AG) via the lipid bilayer (Hurst et al., 2010). In work described here, we used our 2-AG activated CB2 model to produce an initial 2-AG/CB2/Gαi1β1γ2 assembly based on the crystal structure of β2 adrenoreceptor in complex with Gαsβ1γ2 (Rasmussen et al., 2011). Here, the G protein was located underneath CB2, but not inserted into CB2. The 2-AG/CB2/Gαi1β1γ2 assembly was then immersed in a fully hydrated 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer and NPT NAMD (Phillips et al., 2005) molecular dynamics simulations were initiated for four different makes of this system. Initial dynamics (50ns - 150ns) suggests that the C-terminus of Gαi (last 11 residues on α5 helix I344 to F354) inserts itself into the intracellular opening of the CB2 activated receptor at an altered angle compared to the β2 adrenoreceptor/Gαsβ1γ2 complex. The distance between intracellular ends of CB2 TMH3 and TMH6 increases relative to the 2-AG/CB2 complex, suggesting the first step in the formation of the 2-AG/CB2/Gαi1β1γ2 complex. [Support: RO1 DA003934 and KO5 DA021358 (PHR)]